Properties of Murine Pelage Hair Follicles in Monolayer and Collagen Matrix Cultures

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Major aims of hair follicle (HF) research include the elucidation of signals that control the initiation and duration of the hair-cycle phases as well as signals that control differentiation of HF matrix cells to form the various layers of the inner root sheath and of the hair itself. Crucial to these processes are the interaction of dermal papilla cells with HF stem cells in follicle development and with hair matrix cells in the production of hair and associated structures. The challenge to the cell biologist is the creation of an in vitro environment in which the relevant cells continue to perform their in vivo function and can be manipulated in a controlled manner.

We have developed techniques for isolating mouse pelage hair-follicle preparations that differ in their potential to form hair in nude mouse grafts [1]. "Small HF" are epithelial buds just starting their down-growth into the dermis. They remain associated with the epidermis after treatment of skin from 0–2-day-old mice with trypsin. "Standard HF" are prepared essentially as described by Rogers et al [2] with sedimentation through 9% Ficoll replacing filtration through 26 μ Nytex to remove single dermal cells; they represent follicles of intermediate size. "Polytron HF" are obtained by polytron shearing [3] of skin from 3–5-day-old mice. This procedure selects for mature hair follicles with an intact glassy membrane. They are actively producing hair. The results of nude mouse grafts of these follicles led to the following tentative conclusions. "Small HF" are devoid of dermal papilla cells and are absolutely dependent on a fresh dermal cell preparation (collagenase digest of dermis, HF removed) to supply the dermal papilla cells necessary for hair formation. "Standard HF" include within them sufficient numbers of dermal papilla cells to support hair growth in the absence of added dermal cell preparations, but hair growth is enhanced by including dermal cells in the graft. These two HF preparations presumably contain the pluripotent epidermal stem cells [4] required for re-epithelialization of the graft and for hair formation. Because histologic examination of the graft site shows no evidence of HF 1 week after grafting but abundant HF 2 weeks after grafting, it appears that HF form de novo rather than result from continued development of intact HF placed in the graft site. "Polytron HF" fail to produce hairs in grafts, possibly due to the absence of pluripotent stem cells. Furthermore, their dermal papilla cells may be physically constrained from interacting with stem cells, if these are present, to allow for de novo HF formation.

Cells obtained from "polytron HF" by collagenase and trypsin dissociation have very low plating efficiency and grow poorly in monolayer cultures under conditions in which interfollicular epidermal cells and "small HF" form typical cobble-stone epithelial monolayers in 0.05 mM Ca^{2+} medium. "Standard HF" form monolayers under these conditions but show a variety of epithelioid and fibroblastic cell morphologies suggestive of cells that have undergone alteration of their phenotype without losing their proliferative capacity. Some of these cells may represent matrix cells.*

Considering the necessity for interaction between at least two cell types and the complexity of the environment in which HF development and differentiation occur in vivo, a monolayer culture system in simple medium is unlikely to maintain both cell proliferation and specific differentiation. The three-dimensional collagen matrix culture system was therefore adapted for HF [2] to stimulate the in vivo conditions of three-dimensional structure and cell interactions.

In collagen matrix cultures in Medium 199 containing 10% fetal bovine serum [2], "small HF" and "Polytron HF" in particular, but also to some extent "standard HF," undergo changes within 3 to 6 d that are reminiscent of catagen and include loss of basophilic staining of the cells in the central portion of the organoid, decrease in ^{3}H-thymidine incorporation, and decrease in biosynthesis of hair-specific proteins.† In collagen cultures of "standard HF," cholera toxin and epidermal growth factor (EGF) increase and extend the proliferative capacity of HF cells as measured by ^{3}H-thymidine incorporation. In addition, EGF but not cholera toxin was found to increase collagenolytic activity, leading to eventual dissolution of the collagen gel [2]. EGF does not promote the dissolution of the collagen gel in cultures of polytron HF, suggesting that the target cells are either not present or not accessible to the growth factor.

These growth factor studies have been extended to examine the effects of transforming growth factors alpha and beta (TGF-α, TGF-β) on "standard HF" [5]. TGF-α as expected mimicked the effects of EGF. Interestingly, TGF-β inhibited the stimulation of ^{3}H-thymidine incorporation due to EGF or TGF-α but enhanced the elaboration of collagenolytic activity induced by these factors. Collagenolytic activity was attributed to 92-kd and 72-kd precursor type IV collagenases and their activated lower-molecular-weight forms. Because both EGF and TGF-β and their receptors have been shown to be present in the developing HF or surrounding tissue, and because antibody to the 72-kd collagenase stains developing HF in vivo [5], these observations in collagen matrix cultures of HF appear to be relevant to the reorganization of connective tissue required for follicle penetration of the dermis. Furthermore these studies demonstrate the usefulness of the collagen matrix system for examining the effects of various cytokines and other modulators of differentiation alone and in combination.

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Hair Growth Regulation: A Molecular Biologic Approach

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Our goal is to understand the basic molecular mechanisms that regulate hair growth and to develop therapeutic means by which to correct aberrant control in various hair disorders. Toward this end, we have chosen to examine hair keratins as representative molecular markers for switching events associated with hair growth. A major aspect of our initial focus has been the construction of molecular probes suitable for assay of the hair keratins and their genes. More recently, we have begun to analyze the genes themselves, with the hope of eventually being able to utilize hair-specific promoters and other regulatory sequences to target exogenous genes to the hair follicle. We have chosen the mouse as the main model system because of the wide array of relevant mutants with abnormalities of the hair cycle or hair structure, although we are also examining the human hair follicle as our ultimate interest remains human clinical disorders.

Mouse hair keratins [1] fall into two groups: type Ia (acidic; 46–48-K Mr) and type IIa (basic; 58–63 K Mr). Two-dimensional gel electrophoresis further resolves four major members in each of these groups (type Ia, mHa1-4; type IIa, mHb1-4) [2]. The mRNA for these proteins sediment at approximately 18 S and have poly(A)-tails. Hair root poly(A)-containing mRNA were isolated in yields of approximately 3 μg per dorsal skin of 9-day-old C57BL/6J mice. These mRNA were used to prepare a cDNA library. A number of hair-keratin cDNA clones were isolated from this library using partial-length sheep wool keratin probes (a gift from K. Ward, C.S.I.R.O., Australia) for screening. We have focused on four of these clones (Table I) and have found that MHKA-1 and MHKA-2 are full-length type Ia hair-keratin cDNA clones that encode the largest major type Ia hair keratin (mHa1) and the smallest (mHa4), respectively [2]; MHKB-2 encodes a type IIa hair-keratin (mHb4) [3], whereas MHKB-1 is a partial type IIa clone that is still being characterized. Subclones containing 3′-segments from these cDNA have provided specific nucleic acid probes for individual mRNA and genes.

Analysis of deduced amino acid sequences of proteins encoded by the cDNA clones has revealed high similarity among hair-keratin subgroup members. Comparison of corresponding amino acids in mHa1 (416aa) with mHa4 (392aa) indicates that the proteins are strikingly similar overall (92%) with a highly variable carboxy-terminal nonhelical domain (56% identity) [2]. A similar relationship exists among the type IIa keratins. We have utilized these chain-specific differences in C-terminal sequences to prepare monospecific antibodies that recognize individual hair keratins. Antibody AmHa1 was prepared using the C-terminal 14 amino acids of mHa1, the largest of the major mouse type Ia hair keratins, as hapten. Among mouse hair keratin proteins it is monospecific for mHa1 and also recognizes the presumed human counterpart, hHa1. Antibody AmHb4 was prepared similarly using the C-terminal 18 amino acids of mHb4. Thus, amino acid sequence data have enabled us to prepare antibody probes for individual hair keratins, the final gene products.

Specific nucleic acid probes for the hair keratins derived from our clones have permitted chromosomal localization of mouse hair keratin genes [4]. Because the hair and epithelial keratins are homologous in their helical rod domains, our mapping focused on the known chromosomal organization of mouse cytokeratins into two clusters of homologous genes, the Krt-1 locus of type I genes (chromosome 11) and the Krt-2 locus of type II genes (chromosome 15). These keratin loci are tightly linked to mouse mutations affecting skin and hair: Re, Den, and Bsk on chromosome 11 and Ve, Sha, Ca, and N on chromosome 15. DNA from the progeny of C57BL/

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